Bacteria in ornamental fish aquarium water: Are they sensitive to commercial anti-bacterial preparations?

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Abstract

Most bacterial infections in freshwater fish are caused by opportunistic bacteria. Although various commercial antibacterial preparationsare available in the local market, relatively little is known about the efficacy of these preparations and the bacteria present under local conditions. The objectives of the present study were1) to assess the efficacy of some selected (n=7) commercial antibacterial preparations and 2) to isolate and identify bacteria present in aquarium water. Commercial preparations containing formalin (A), acriflavine (B and C), flavin (D), methyleneblue (E), potassium permanganate (F), and iodine (G), as the active ingredient, were tested in this study. Water samples (n = 9) were collected aseptically from fish-tanks at the university aquarium, during a period of 3 days. Fifty microliters from each sample was cultured separately on nutrient agar and then one replicate of each was treated with one antibacterial preparation separately, at their manufacturer recommended dose (MRD). As for F and G, trial doses were used. Treated samples were kept at room temperature after mixing well. Sub-samples were collected from each treatment at 10 min, 30 min and 1hr and were cultured separately by spread plate method. All cultures were incubated at 37°C, overnight. The bacterial colonies were then enumerated using the colony count (Rocker, Taiwan). The total plate count (CFU/ml) dropped significantly ($p<0.05$, n=3) 10 minutes after the treatment with potassium permanganate (7.04±2.13%) and dropped further at 30 minutes (3.22±1.16%) and at 1 hour (2.64±1.19%), compared with the control (100%). The acriflavine and flavin preparations (B, C and D) showed differences in antibacterial activity. Anti-bacterial efficacy of the products carrying methylene blue and formalin preparations were not significant ($p>0.05$) at MRD. Potassium permanganate demonstrated the most promising anti-bacterial efficacy out of all the preparations tested. *Pseudomonas aeruginosa* and Bacillus spp. could be identified from the samples.

Keywords: aquarium water, anti-bacterial preparations, bacteria, efficacy, ornamental fish

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Introduction

Colorful aquaria are maintainedforrecreation and as an enterprise. It is important to maintain ornamental fish under stress-free conditions, in order to maintain fish health. Most of the pathogenic bacteria in freshwater fish areopportunistic (Austin and Austin, 2007).Formalin, malachite green, potassium permanganate,acriflavine and methylene

blue are some of the most commonly used antibacterial compounds for aquarium tanks (Maity et al, 2011).Although various commercial antibacterial preparations are available in the local market, relatively little is known about the efficacy of these preparations and the bacteria present under local conditions. Thus the objectives of the present study were 1) to assess the efficacy of some selected (n=7) commercial antibacterial preparations and 2) to isolate and identify bacteria present in aquarium water.

Materials and Methods

The commercial preparations containing formalin (A),acriflavine(B and C), flavin (D),methyleneblue (E),potassium permanganate (F),and iodine (G, were tested in this study.Water samples(n = 9) were collected aseptically, from fish-tanks at the university aquarium, during a period of 3 days. Fifty microliters from a sample on each day was cultured directly on nutrient agar and then one replicate each was treated with one antibacterial preparation separately, at their manufacturer recommended dose (MRD), on each day. As for F and G, treatment doses were decided based on a trial-and-error methods and existing literature. Three different doses were tested separately, for F and G. Anti-bacterially treated samples were kept at room temperature after mixing well. Sub-samples were collected from each treatment at 10 min, 30 min and 1hr and were cultured separately by spread plate method. An additional sub-sample was collected from the replicate treated with A, after 3h, for spread plating method. All cultures were incubated at 37^0C, overnight. The bacterial colonies were then enumerated using the colony count (Rocker, Taiwan). Colony count (CFU/ml) of control samples were compared with that of sub-samples collected from different treatment regimens and different time intervals, were compared using generalized linear models (GENLIN) of SPSS version 20.0 (IBM Corporation, Somers, NY, USA).

Furthermore, the colonial morphology of different isolates on nutrient agar was studied. Each different colony was subjected to microscopic examination of Gram-stained smears. Subsequently colonies were sub-culturedonMacConkey agar (Himedia, India) and Pseudomonas selective agar (Himedia, India), based on colonial morphology and microscopicappearance. Finally, biochemical tests were performed to identify the genus/species of each type.

Results and Discussion

At the most effective dose, the total plate count (CFU/ml) dropped significantly ($p<0.05$, n=3) 10 minutes after the treatment with potassium permanganate (7.04±2.13%) and dropped further at 30 minutes (3.22±1.16%) and at 1 hour

(2.64±1.19%), compared with the control (100%).The study revealed an effective dosage of100µl/L of water, for potassium permanganate.Theacriflavine and flavin preparations (B, C and D)showed differences in antibacterial activity.Anti-bacterial efficacy of the products carrying methylene blue and formalin preparations were not significant ($p>0.05$) at MRD.The antibacterial action of Lugol's Iodine was also significant($p<0.05$, $n=3$)10 minutes after the treatment (18.14±4.66%), and dropped further after 30 minutes (6.78±5.58%). However, there was rise (12.48±3.98%) after 1 hour from treatment, compared to the count after 30 minutes post-treatment. Lugol's iodine is not usually used to treat bacterial infections in fish. However, previous studies also have reported safe elimination of surface bacteria in fish by using Lugol'siodine.*Pseudomonas aeruginosa* and Bacillus spp. could be identified from the samples.

Conclusion

Potassium permanganate demonstrated the most promising anti-bacterial efficacy out of all the preparations tested. The study revealed an effective dosage of100µl/L of water, for potassium permanganate. *Pseudomonas aeruginosa* and Bacillus spp. could be identified from the samples.

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